THE EFFECT OF TOLUENE ON THE PRODUCTION OF ANTIBODIES *

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In connection with experiments on the effects of benzene on the production of antibodies, experiments of a similar nature were made with toluene. The preparation used was "Toluene Merck-Pure." Practically the same methods were followed as in the work on benzene, and the titers of the antibodies studied were determined as described in the report of that work.[1]

The first experiment was designed to test the action of toluene injected subcutaneously on the 4th day after an intraperitoneal injection of 30 c.c. of sheep blood, and daily for 8 days thereafter. The 3 rabbits used and the control were of the same litter, and weighed about 1500 gm. each. The daily quantity of toluene in each case was

TABLE 1

THE FORMATION OF LYSIN AND PRECIPITIN IN RABBITS INJECTED WITH TOLUENE DAILY FOR 8 DAYS, BEGINNING WITH THE 4TH DAY AFTER THE INJECTION OF SHEEP BLOOD

Days After Injection of Sheep Blood	Injections of Toluene	Rabbit 1		Rabbit 2		Rabbit 3		Control (no toluene)	
		Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin
2		768	0	384	0	768	0	768	0
4		12288	0	3072	0	384	0	768	0
5	+								
6	+	24576	180	24576	180	12288	180	6144	50
7	+								
8	+	12288	1800	12288	1800	6144	200	6144	800
9	+	12288	1800	12288	1800	6144	50	6144	1600
10	+								
11	+	12288	1800	12288	1800	6144	80	12288	3200
12	+	6144	1800	3072	1800	1536	50	12288	6400
13		6144	1800	6144	1800	1536	40	6144	12800
15		Died		6144	1800	3072	25	3072	12800
16				6144	1800	3072	0	3072	12800
17				6144	1800	1536		3072	12800
20				6144	1800	768		3072	12800
23				6144	1800	768		3072	12800
28				1536	800	768		3072	12800
36				3072	400	768		6144	3200
42				3072	50	768		1536	1600
49				1536		768		1536	800
58				192		48		96	200

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[1] Jour. Infect. Dis., 1916, 19, p. 69.

TABLE 2

THE EFFECT ON THE PRODUCTION OF LYSIN AND PRECIPITIN OF 12 DAILY INJECTIONS OF 1 C.C. OF TOLUENE PER KILO OF WEIGHT, THE FIRST ON THE SAME DAY THAT 30 C.C. OF SHEEP BLOOD WERE INJECTED INTRAPERITONEALLY

Days After Injection of Sheep Blood	1		2		3	
	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin
1	96		192		24	
2	95		384		24	
3	192		768		24	
4	1536	10	1536	10	768	
5	24576	100	12288	100	6144	25
6	24576	100	12288	100	12288	100
7	12288	600	12288	600	12288	400
8	12288	1200	12288	1200	12288	3200
9	12288	1200	12288	1200	6144	3200
10		1200	12288	1200	6144	3200
11	24576	1200	6144	2400	6144	3200
12	24576	2400	12288	2400	6144	6400
13	12288	2400	12288	4800	6144	6400
14	12288	2400	12288	4800	3072	6400
15	12288	2400	12288	2400	3072	12800
16	12288	2400	12288	2400		
17					1536	3200
18	12288	2400	12288	2400		
19					1536	3200
20	12288	2400	12288	2400		
22	12288	2400	12288	2400		
24					768	800
25	12288	2400	12288	2400		
26						
28						
30	6144	2400	3072	2400		
31					1536	800
35	6144	2400	3072	2400		
38					768	800
40	12288	2400	1536	9600		
43						
45					768	800
47	6144	6400	768	16800		
50						
52					768	400
54	12288	6400	384	6400	Discharged	
58						
61	6144	3200	384	12800		
64						
66						
68	12288	3200	384	12800		
75	6144	1280	384	10240		
82	3072	3200	384	12800		
85						
87						
89	6144	3200	384	12800		
94						
99	3072	6400	192	12800		
101	3072	6400	192	12800		
117	1536	9600	384	9600		
122	6144	2400	1536	19200		
132	6144	9600	197	160000		
148						
162	1536	9600	384	9600		
170	1536	9600	384	9600		
178	1536	9600	384	25600		
188	768	4800	384	12800		
198	1536	4800	192	7700		
205	1536	4800	192	9600		
212	1536	4800		4800		
231	768	800	768	400		
237	768	400	384	800		
243	Died		192	200		

Rabbits 1 and 2 received 1-1.5 c.c. benzene subcutaneously daily from the 96th to the 109th day.

TABLE 2—*Continued*

THE EFFECT ON THE PRODUCTION OF LYSIN AND PRECIPITIN OF 12 DAILY INJECTIONS OF 1 C.C. OF TOLUENE PER KILO OF WEIGHT, THE FIRST ON THE SAME DAY THAT 30 C.C. OF SHEEP BLOOD WERE INJECTED INTRAPERITONEALLY

4		5		Control (no toluene)		Control (no toluene)	
Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin
						384	
						768	25
6144		6144		12288	400	12288	200
49152	400	12288	100	12288	1600	24576	1600
						49152	3200
12288	800	12288	800			49152	3200
				49152	25600		
						49152	9600
6144	2400	6144	1200				
				49152	12800	49152	4800
12288	4800	12288	1200				
						24576	4800
12288	9600	12288	2400	49152	6400		
						24576	1600
	19200	3072	9600				
				24576	6400		
						6144	3200
1536	6400	1536	3200	12288	4800		
1536	4800	3072	2400			1536	800
				6144	4800		
768	12800	384	3200	3072	4800		
384	9600	384	3200				
						768	200
768	9600	768	3200	3072	4800		
768	9600	384	4800	1536	400		
				1536	200		
384	19200	384	4800				
						768	200
384	9600	384	1200	1536	200		
				1536	160		
192	4800	768	4800			768	200
192	2400	1536	4800	1536	0	768	0
				Discharged		768	0
384	300	1536	3200			Discharged	
192	12800	384	6400				
384	800	384	600				
Discharged		384	0				
		192	0				
		96					
		Discharged					

1 c.c. in 1 c.c. of olive oil. No change in the number of leukocytes in the blood was observed and the general health of the animals remained good, tho one rabbit died after 2 weeks. The results as to the production of lysin and precipitin are given in Table 1. The principal effect of the toluene appears to have been a reduction in the amount of precipitin.

In the next experiment the injection of toluene was commenced on the same day as the injection of sheep blood. Five rabbits, weighing about 1000 gm. each, were given 30 c.c. of sheep blood intraperitoneally and 1 c.c. of toluene subcutaneously. The injections of toluene were repeated each day for 12 days. No definite change resulted in the number of leukocytes. The results as to lysin and precipitin are shown in Table 2. While there may have been some restraint in the early production of antibodies, the outstanding feature was the persistence of lysin and precipitin longer and with greater fluctuation in most of the toluene rabbits than in the controls (rabbits injected with the same amount of sheep blood, but not with toluene). That the daily bleeding of some of these rabbits in the early period of antibody-production had some influence on the output of antibodies cannot be excluded, but that the later fluctuation and persistence of the lysin and precipitin resulted wholly from the effects of the bleedings cannot be maintained, because similar deviations from the usual course occurred in experiments with toluene in which the bleedings were not so frequent; and it may be pointed out too that so far as my observations go the removal of large amounts of blood from recently immunized animals does not disturb the typical curve of the antibody course in the blood, but tends rather to increase the antibody output. Hence it probably is justifiable to ascribe the unusual fluctuation and persistence of lysin and precipitin in the toluenized rabbits to the action of toluene combined perhaps with the effects of daily bleedings during the first two weeks after the injection of antigen.

Rabbits 1 and 2, Table 2, require special consideration. It is noteworthy that in these animals there occurred an increase in the titer of the precipitin as late as 40-50 days after the injection of the antigen and a persistent but fluctuating high titer of both lysin and precipitin thereafter for a long period. From the 96th to the 109th day they received daily, at first 1 c.c., and then 1.5 c.c. of benzene subcutaneously, and, as mentioned in the article on benzene, seemingly without any immediate effects either on the leukocytes, the general

health of the animals, or on the course of the lysin and precipitin. This general result is in harmony with the results of the injection of benzene at the height of antibody-production in rabbits not previously injected with toluene.[1] Since in the latter animals the production of lysin and precipitin persisted longer and underwent more fluctuation than under ordinary circumstances, it is altogether reasonable to conclude that the treatment with benzene of the toluenized rabbits, 1 and 2, tended to increase still further the persistence and fluctuation of the lysin and precipitin. Certainly the persistence of these antibodies in the concentration shown in the table for some 243 days after the injection of the antigen forms a noteworthy deviation from the usual course of antibodies in general and of antisheep precipitin in particular.

Experiments designed to test the action of toluene injected for some days before the introduction of antigen may be summarized as follows: Five rabbits received daily 1 c.c. of toluene per kilo of weight for from 10 to 15 days before the injection of sheep blood. All save

TABLE 3

THE EFFECT ON ANTIBODY-PRODUCTION OF DAILY INJECTIONS OF TOLUENE, 1 C.C. PER KILO OF WEIGHT, FOR FROM 10 TO 15 DAYS BEFORE INJECTION OF SHEEP BLOOD

Days After Injection of 30 c.c. Sheep Blood	1		2		3		4		5	
	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin	Lysin	Precipitin
1					0		0		0	
2					0		0		96	
3					768	0	768	0	1536	0
4	48	0	1536	0	12288	50	12288	50	12288	50
5					6144	100	12288	100	12288	100
7	1536	0	12288	800	24576	400	24576	400	24576	600
8					12288	400	12288	800	24576	800
9					24576	400	24576	800	24576	800
10	3072	0	12288	6400	24576	400	24576	400	24576	800
13	3072	0	12288	6400	12288	800	12288	1200	24576	1200
15					12288		6144	2400	6144	1200
16	3072	0	6144	6400						
18					6144	600	3072	600	6144	400
20	768	0	1536	3200						
22					6144	400	3072	600	6144	1200
23	1536	0	1536	12800						
27	768	0	1536	200000						
31	768	0	768	0						
34	768	0	768	0						
35			Died		3072	200	1536	400	3072	800
38	384	0								
45	192	0			768	100	536	200	3072	800
55	384	0			Died		Died		1536	400
64	384	0							Died	
69	192	0								
85	384	0								
94	384	0								
105	384	0								

one died without obvious cause before the production of antibodies had run its course. The survivor, Rabbit 1, Table 3, gave no precipitin at all, and only a small amount of lysin, probably because of an inherent lack of power to produce antibodies. Rabbit 2, Table 3, of another series, which like Rabbit 1 received toluene for 10 days, approached the normal average of lysin and precipitin, but the very high concentration of precipitin on the 27th day, followed by an apparent total loss of this antibody, is remarkable and exceptional. Rabbits 3, 4, and 5, Table 3, which received toluene for 15 days, produced about as much lysin as normal rabbits but not nearly as much precipitin. Taken altogether, these results indicate that prolonged toluene treatment antecedent to the injection of antigen may reduce the output of precipitin.

To test the effect of toluene when introduced in the course of antibody-formation, injections of toluene were commenced on the 12th day after 30 c.c. of sheep blood had been injected. Table 4 illustrates the procedure as well as the results. It appears that lysin was reduced for a few days, but it is not evident that precipitin was affected. However, it is noteworthy that the titers of lysin and precipitin remained well above normal for some 200 days, far beyond the limit in untreated rabbits.

The effects of smaller doses of toluene have also been studied but not extensively. The subcutaneous injection of 0.01 c.c. of toluene per kilo of weight each day for 12 days, beginning on the same day that sheep blood was injected intraperitoneally, had no depressive effect on the production of lysin and precipitin. The rabbits (4) all died about the 80th day after the injection of sheep blood, the lysin titers then being 1536, 768, 1536, and 192, and the corresponding precipitin titers 3200, 400, 800, and 1600. These values, especially those of the precipitin, are higher than is usually the case at the same period in rabbits which have been injected with sheep blood only.

The injection of 0.005 c.c. of toluene per kilo of weight daily for 4 days before, and 10 days after, the injection of sheep blood had no appreciable effect on antibody-production so far as could be observed, but the rabbits (3) all died within 50 days of the introduction of the blood.

This high death rate of the rabbit has been a serious handicap, especially in that it is necessary to keep the animals under observation over long periods. This is one reason why the results now presented are somewhat fragmentary and inconclusive.

TABLE 4

THE EFFECT OF TOLUENE WHEN INJECTED AT THE HEIGHT OF ANTIBODY-PRODUCTION

Days After Injection of 30 c.c. of Sheep Blood	Injections of Toluene, c.c.	Lysin	Precipitin
1		0	
2		0	
3		96	
4		3072	
5		24576	100
6		24576	600
7		12288	600
8		24576	600
10		24576	2400
11		12288	2400
12	0.3	6144	2400
13	0.3	6444	2400
15	0.5	768	4800
17	0.5	768	3200
18	0.5	768	3200
20	0.5	768	3200
22	0.75	3072	3200
25	0.75	1536	2400
28	0.75	1536	1600
32	0.75	1536	1200
35		3072	2400
42		1536	2400
50		1536	3200
59		768	2400
70		768	1200
77		1536	1200
84		1536	3200
94		768	1600
103		768	400
113		768	800
129		384	800
145		384	400
161		384	200
174		192	400
186		384	200
203		384	50
216		192	0

The results of the experiments indicate that toluene injected into rabbits as outlined in this report reduces the output of antibody in the blood, especially of precipitin, during the early stages of antibody-production; under certain circumstances, however, as when injected while the antibody content of the blood is at its height, or as shown in Table 2, toluene appears to cause greater late fluctuation and longer persistence of new antibody than occur under ordinary conditions. Even relatively small doses of toluene may have effects of this kind. As already explained, it does not seem likely that such effects, which are obtainable with benzene also, can result from repeated bleedings.

A few facts and considerations bearing on the question of how toluene produces its effects may be discussed briefly. It might be said that toluene in some way interferes with the absorption and fixation of the antigen, but so far as the results of

observations with the precipitin method go, the course of the antigen —sheep protein—in the blood appears to be quite the same in rabbits injected with toluene (and benzene[1]) as in rabbits not so injected. Furthermore, the fact that toluene injected as late as 12 days after the introduction of antigen appears to prolong greatly the period of persistence of antibody in the blood (Table 4), speaks against the assumption of a delay and change in the absorption and fixation of antigen.

In the course of this work I confirmed the results of Kline and Winternitz[2] that toluene in the quantity of 1 c.c. per kilo does not affect the number and proportion of leukocytes in the blood of rabbits, at least in the early stages after its introduction. No observations were made in the later stages. The phagocytic activity in vitro of the leukocytes of toluenized rabbits did not appear to be reduced—a circumstance which is in harmony with the observation of Kline and Winternitz that there is no difference in response to experimental pneumococcus infection between rabbits treated with toluene and normal rabbits. Likewise the observation of Kline and Winternitz that the marrow of rabbits injected with toluene (1 c.c. per kilo) presents a hyperplasia, especially of the myeloid cells, there being only slight changes in the spleen and lymph glands, is confirmed by the results obtained incidentally to the study of the effects of toluene on antibody-formation. The most marked changes seemed to develop in the marrow of the femur, the next most marked in that of the humerus, while in that of the tibia, ulna, radius, ribs, and spinal column the changes were only slight. The myelocytes played the chief rôle in this toluene hyperplasia of the marrow, the nongranular and amphophil cells being far in excess of other kinds of cells. The hyperplasia subsided quickly when the injection of toluene was stopped, so that in about 6 days the number of cells was normal again. From then on, the number of cells in the marrow of the toluenized rabbits appeared to be smaller than normal, the cells being replaced by a homogeneous substance with but few fat cells. During the period of hyperplasia the giant cells of the marrow seemed more actively phagocytic for leukocytes than under normal conditions. There were no noteworthy changes attributable to the effects of toluene in any of the other tissues of the body.

Toluene, then, has a direct effect on cells in the marrow, which, as

[2] Jour. Exper. Med., 1914, 18, p. 50.

developed more fully in the report on benzene, there are many reasons to believe are concerned directly with the elaboration of antibodies. As this effect is observed only for a short time after the injection of toluene, it may be the explanation of why toluenized rabbits produce less antibodies than normal rabbits in the earlier stages of antibody-formation. On the other hand, as the abnormal fluctuation and persistence of antibody in toluenized rabbits occur at a time when the cells of the marrow, if anything, are fewer in number than normally, we are left without any apparent morphologic basis for an explanation of these interesting phenomena. Is there in such animals a late increase in the production of antibodies, or are conditions established that interfere with the passage of accumulated antibodies out of the blood? This and other questions can be answered only by further investigation.

SUMMARY

In rabbits the effect of toluene in repeated doses of about 1 c.c. per kilo of weight is to diminish the output of antibody in the earlier periods of antibody-production, and, under certain conditions, to cause prolonged persistence of antibody in the blood, with, in at least some cases, marked fluctuations in the concentration.

Increased persistence of antibody in the blood may follow smaller doses of toluene also.

There is no immediate change as to number and proportion of leukocytes in the blood of rabbits treated with toluene as here described, nor is there any change in the phagocytic activity of the leukocytes in vitro.

In rabbits receiving the larger doses of toluene there occurs a transitory myeloid hyperplasia in the bone marrow, but later no marked changes in the marrow or other organs appear. On this account there is at present no basis for concluding whether the fluctuation and persistence of antibodies in the blood is better explained as resulting from increased and prolonged production of antibodies, or as resulting from interferences with the passage of antibodies out of the blood.